

Best Available Copy

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A01N 43/38, A61K 31/40		A1	(11) International Publication Number: WO 00/40088 (43) International Publication Date: 13 July 2000 (13.07.00)
<p>(21) International Application Number: PCT/US99/30220</p> <p>(22) International Filing Date: 16 December 1999 (16.12.99)</p> <p>(30) Priority Data: 09/226,693 7 January 1999 (07.01.99) US</p> <p>(71) Applicant: VANDERBILT UNIVERSITY [US/US]; Office of Technology Transfer, Suite 210, 1207 17th Avenue South, Nashville, TN 37212 (US).</p> <p>(72) Inventors: KALGUTKAR, Amit, S.; 111 Acklen Park Drive, A-4, Nashville, TN 37203 (US). MARNETT, Lawrence, J.; 1884 Laurel Ridge Drive, Nashville, TN 37215 (US).</p> <p>(74) Agent: TAYLOR, Arles, A., Jr.; Jenkins & Wilson, P.A., University Tower, Suite 1400, 3100 Tower Boulevard, Durham, NC 27707 (US).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: AMIDE DERIVATIVES FOR ANTIANGIOGENIC AND/OR ANTITUMORIGENIC USE</p> <p>(57) Abstract</p> <p>A method of treating animals having cancer by administration of secondary amide derivatives of various COOH-containing drugs, such as COOH-containing NSAIDs, for instance, indomethacin.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

DescriptionAMIDE DERIVATIVES FOR ANTIANGIOGENIC AND/OR
ANTITUMORIGENIC USEGovernment Interest

5 This research was funded by a research grant from the National Institutes of Health (Research Grant No. CA47479). Thus, the United States government has certain rights in the invention.

Technical Field

The present invention, in general, relates to conversion of the carboxylic acid moiety of various compounds into amide derivatives of the compounds. More specifically, the present invention relates to secondary amide derivatives of non-steroidal antiinflammatory drugs (NSAIDs), particularly of indomethacin (an NSAID), that exhibit inhibition of cyclooxygenase-2 (COX-2) far exceeding inhibition of cyclooxygenase-1 (COX-1), and also, that still exhibit the analgesic, antiinflammatory, and/or antipyretic effect of the compound, i.e., of the NSAID, and moreover, also exhibit cancer inhibition, i.e., an antiangiogenic

-2-

and/or antitumorigenic effect, in warm blooded vertebrate animals, including humans.

Table of Abbreviations

	<u>Abbreviations</u>	<u>Definitions</u>
5	NSAID	non-steroidal antiinflammatory drug
	COOH	carboxylic acid moiety
	COX	cyclooxygenase
	PGH ₂	prostaglandin H ₂
	PGD ₂	prostaglandin D ₂
	PGHS	prostaglandin endoperoxide synthase
10	PER	peroxidase
	SAR	structure-activity relationship
	GI	gastrointestinal
	IC ₅₀	concentration in micromoles of indomethacin (or the indomethacin derivative) at which there is 50% inhibition of COX activity—the lower the IC ₅₀ is, then the more potent the drug is
15	DMSO	dimethyl sulfoxide
	¹⁴ C-AA	[1- ¹⁴ C]-arachidonic acid
	HPLC	high performance liquid chromatography
	TLC	thin layer chromatography

	mg	milligram
	kg	kilogram
	mL	milliliter
	µM	micromole/liter
5	µL	microliter
	N	normal (when used in conjunction with acid concentrations)
	NMR	nuclear magnetic resonance
	Et ₂ O	diethyl ether
10	EtOAc	ethyl acetate
	Et ₃ N	triethyl amine
	AcOH	acetic acid
	CDCl ₃	deuteriated chloroform
	rt	room temperature (about 72°F, 22°C)
15	BOP-Cl	bis(2-oxo-3-oxazolidinyl)phosphonic chloride (sold by Aldrich in Wisconsin), and also see the journal article, Diago-Meseguer, Palomo-Coll, Fernandez-Lizarbe, and Zugaza-Bilbao, "New Reagent for Activating Carboxyl Groups; Preparation and Reactions of N,N'-Bis[2-oxo-3-oxazolidinyl] phosphorodiamidic Chloride", <i>Synthesis</i> (1980) pp. 547-551
20		
25		
	mp	melting point
	FBS	fetal bovine serum
	DMEM	Dulbecco's modified essential medium

-4-

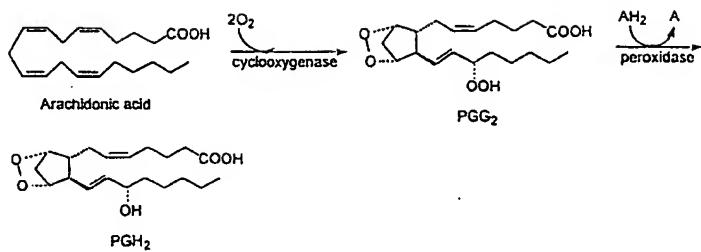
LPS	lipopolysaccharide
PBS	phosphate-buffered saline
IFN-g	interferon gamma

Background of the Invention

5 As discussed in more detail below, the COX enzyme is really two enzymes, COX-1 and COX-2, which serve different physiological and pathophysiological functions. As is well known, at antiinflammatory and/or analgesic doses, indomethacin, aspirin, and other NSAIDs effect great inhibition of COX-1, which protects the lining of the stomach from acid, along
10 with relatively minimal inhibition of COX-2, which provokes inflammation in response to joint injury or a disease like arthritis. Also, certain NSAIDs possess essentially the same inhibitory activity against both COX-1 and COX-2. Thus, zeroing in on inhibition of COX-2 alone has been the goal of drug developers for several years in order to reduce or eliminate the GI irritation caused by
15 COX-1 inhibition.

More specifically, as discussed in Smith, Garavito, and DeWitt, "D.L. Prostaglandin Endoperoxide H Synthases (Cyclooxygenases) -1 and -2", *J. Biol. Chem.*, (1996) Vol. 271, pp. 33157-33160, the pertinent step in prostaglandin and thromboxane biosynthesis involves the conversion of
20 arachidonic acid to PGH₂, which is catalyzed by the sequential action of the COX and PER activities of PGHS, as set out in the following reaction scheme:

-5-



That COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2, is described in DeWitt and Smith, "Primary Structure of Prostaglandin G/H Synthase from Sheep Vesicular Gland Determined from the Complementary DNA Sequence", *Proc. Natl. Acad. Sci. U.S.A.* (1988) Vol. 85, pp. 1412-1416; Yokoyama and Tanabe, "Cloning of Human Gene Encoding Prostaglandin Endoperoxide Synthase and Primary Structure of the Enzyme", *Biochem. Biophys. Res. Commun.* (1989) Vol. 165, pp. 888-894; and Hla and Neilson, "Human Cyclooxygenase-2-cDNA", *Proc. Natl. Acad. Sci. U.S.A.* (1992) Vol. 89, pp. 7384-7388.

COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins in the GI tract and for the synthesis of thromboxane, which triggers platelet aggregation in blood platelets. See, Allison, Howatson, Torrence, Lee, and Russell, "Gastrointestinal Damage Associated with the Use of Nonsteroidal Antiinflammatory Drugs", *N. Engl. J. Med.* (1992) Vol. 327, pp. 749-754.

On the other hand, COX-2 is inducible and short-lived. Its expression is stimulated in response to endotoxins, cytokines, and mitogens. See, Kujubu,

-6-

Fletcher, Varnum, Lim, and Herschman, "TIS10, A Phorbol Ester Tumor Promoter Inducible mRNA from Swiss 3T3 Cells, Encodes a Novel Prostaglandin Synthase/Cyclooxygenase Homologue", *J. Biol. Chem.* (1991) Vol. 266, pp. 12866-12872; Lee, Soyoola, Chanmugam, Hart, Sun, Zhong,
5 Liou, Simmons, and Hwang, "Selective Expression of Mitogen-Inducible Cyclooxygenase in Macrophages Stimulated with Lipopolysaccharide", *J. Biol. Chem.* (1992) Vol. 267, pp. 25934-25938; and O'Sullivan, Huggins, Jr., and McCall, "Lipopolysaccharide-Induced Expression of Prostaglandin H Synthase-2 in Aveolar Macrophages is Inhibited by Dexamethasone but not by Aspirin",
10 *Biochem. Biophys. Res. Commun.* (1993) Vol. 191, pp. 1294-1300.

Importantly, COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages) and in the central nervous system. See, Masferrer, Zweifel, Manning, Hauser, Leahy, Smith, Isakson, and Seibert, "Selective Inhibition of Inducible Cyclooxygenase-2 *in vivo* is
15 Antiinflammatory and Nonulcerogenic", *Proc. Natl. Acad. Sci. U.S.A.* (1994) Vol. 91, pp. 3228-3232; Vane, Mitchell, Appleton, Tomlinson, Bishop-Bailey, Croxtall, and Willoughby, "Inducible Isoforms of Cyclooxygenase and Nitric Oxide Synthase in Inflammation", *Proc. Natl. Acad. Sci. U.S.A.* (1994) Vol. 91, pp. 2046-2050; Harada, Hatanaka, Saito, Majima, Ogino, Kawamura, Ohno,
20 Yang, Katori, and Yamamoto, "Detection of Inducible Prostaglandin H Synthase-2 in Cells in the Exudate of Rat Carrageenin-Induced Pleurisy", *Biomed. Res.* (1994) Vol. 15, pp. 127-130; Katori, Harada, Hatanaka, Kawamura, Ohno, Aizawa, and Yamamoto, "Induction of Prostaglandin H

Synthase-2 in Rat Carrageenin-Induced Pleurisy and Effect of a Selective COX-2 Inhibitor", *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (1995) Vol. 23, pp. 345-347; and Kennedy, Chan, Culp, and Cromlish, "Cloning and Expression of Rat Prostaglandin Endoperoxide 5 Synthase (Cyclooxygenase-2) cDNA", *Biochem. Biophys. Res. Commun.* (1994) Vol. 197, pp. 494-500.

Hence, the differential tissue distribution of COX-1 and COX-2 provides a basis for the development of drugs that are selective COX-2 inhibitors (i.e., specificity for inhibition of COX-2 far exceeds inhibition of COX-1) as 10 antiinflammatory, analgesic, and/or antipyretic agents with minimization of or without the GI and hematologic liabilities from COX-1 inhibition that plague most all currently marketed NSAIDs, most of which inhibit both COX-1 and COX-2, with specificity for COX-1 inhibition greatly exceeding that for COX-2 inhibition, although some have essentially similar inhibitory activity against both 15 COX-1 and COX-2. See, for instance, Meade, Smith, and DeWitt, "Differential Inhibition of Prostaglandin Indoperoxide Synthase (Cyclooxygenase) Isozymes by Aspirin and Other Non-Steroidal Antiinflammatory Drugs", *J. Biol. Chem.*, (1993) Vol. 268, pp. 6610-6614.

Detailed SAR studies have been reported for two general structural 20 classes of selective COX-2 inhibitors (specificity for COX-2 inhibition far exceeds COX-1 inhibition) including certain acidic sulfonamides and diarylheterocyclics. The *in vivo* activities of these selective COX-2 inhibitors validate the concept that selective COX-2 inhibition is antiinflammatory and

nonulcerogenic, as discussed in the following journal articles. Gans, Galbraith, Roman, Haber, Kerr, Schmidt, Smith, Hewes, and Ackerman, "Anti-Inflammatory and Safety Profile of DuP 697, a Novel Orally Effective Prostaglandin Synthesis Inhibitor", *J. Pharmacol. Exp. Ther.* (1990) Vol. 254, 5 pp. 180-187; Penning, Talley, Bertenshaw, Carter, Collins, Docter, Graneto, Lee, Malecha, Miyashiro, Rogers, Rogier, Yu, Anderson, Burton, Cogburn, Gregory, Koboldt, Perkins, Seibert, Veenhuizen, Zhang, and Isakson, "Synthesis and Biological Evaluation of the 1,5-Diarylpyrazole Class of Cyclooxygenase-2 Inhibitors: Identification of 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, Celecoxib)", *J. Med. Chem.* (1997) Vol. 40, pp. 1347-1365; Khanna, Weier, Yu, Xu, Koszyk, Collins, Koboldt, Veenhuizen, Perkins, Casler, Masferrer, Zhang, Gregory, Seibert, and Isakson, "1,2-Diarylimidazoles as Potent Cyclooxygenase-2 Selective, and Orally Active Antiinflammatory Agents", *J. Med. Chem.* (1997) 10 Vol. 40, pp. 1634-1647; Khanna, Weier, Yu, Collins, Miyashiro, Koboldt, Veenhuizen, Curie, Siebert, and Isakson, "1,2-Diarylpyrroles as Potent and Selective Inhibitors of Cyclooxygenase-2", *J. Med. Chem.* (1997) Vol. 40, pp. 1619-1633; Tsuji, Nakamura, Konishi, Tojo, Ochi, Senoh, and Matsuo, "Synthesis and Pharmacological Properties of 1,5-Diarylpyrazoles and Related 15 Derivatives", *Chem. Pharm. Bull.* (1997) Vol. 45, pp. 987-995; Riendeau, Percival, Boyce, Brideau, Charleson, Cromlish, Ethier, Evans, Falgueyret, Ford-Hutchinson, Gordon, Greig, Gresser, Guay, Kargman, Léger, Mancini, O'Neill, Quellet, Rodger, Thérien, Wang, Webb, Wong, Xu, Young, Zamboni, 20

-9-

Prasit, and Chan, "Biochemical and Pharmacological Profile of a Tetrasubstituted Furanone as a Highly Selective COX-2 Inhibitor", *Br. J. Pharmacol.* (1997) Vol. 121, pp. 105-117; Roy, Leblanc, Ball, Brideau, Chan, Chauret, Cromlish, Ethier, Gauthier, Gordon, Greig, Guay, Kargman, Lau,
5 O'Neill, Silva, Thérien, Van Staden, Wong, Xu, and Prasit, "A New Series of Selective COX-2 Inhibitors: 5,6-Diarylthiazolo[3,2-b][1,2,4]-triazoles", *Bioorg. Med. Chem. Lett.* (1997) Vol. 7, pp. 57-62; Thérien, Brideau, Chan, Cromlish, Gauthier, Gordon, Greig, Kargman, Lau, Leblanc, Li, O'Neill, Riendeau, Roy, Wang, Xu, and Prasit, "Synthesis and Biological Evaluation of 5,6-
10 Diarylimidazo[2.1-b]thiazoles as Selective COX-2 Inhibitors", *Bioorg. Med. Chem. Lett.* (1997) Vol. 7, pp. 47-52; Li, Norton, Reinhard, Anderson, Gregory, Isakson, Koboldt, Masferrer, Perkins, Seibert, Zhang, Zweifel, and Reitz, "Novel Terphenyls as Selective Cyclooxygenase-2 Inhibitors and Orally Active Anti-Inflammatory Agents", *J. Med. Chem.* (1996) Vol. 39, pp. 1846-1856; Li,
15 Anderson, Burton, Cogburn, Collins, Garland, Gregory, Huang, Isakson, Koboldt, Logusch, Norton, Perkins, Reinhard, Seibert, Veenhuizen, Zhang, and Reitz, "1,2-Diarylcyclopentenes as Selective Cyclooxygenase-2 Inhibitors and Orally Active Anti-Inflammatory Agents", *J. Med. Chem.* (1995) Vol. 38, pp. 4570-4578; Reitz, Li, Norton, Reinhard, Huang, Penick, Collins, and Garland,
20 "Novel 1,2-Diarylcyclopentenes are Selective Potent and Orally Active Cyclooxygenase Inhibitors", *Med. Chem. Res.* (1995) Vol. 5, pp. 351-363; Futaki, Yoshikawa, Hamasaka, Arai, Higuchi, Iizuka, and Otomo, "NS-398, A Novel Nonsteroidal Antiinflammatory Drug with Potent Analgesic and

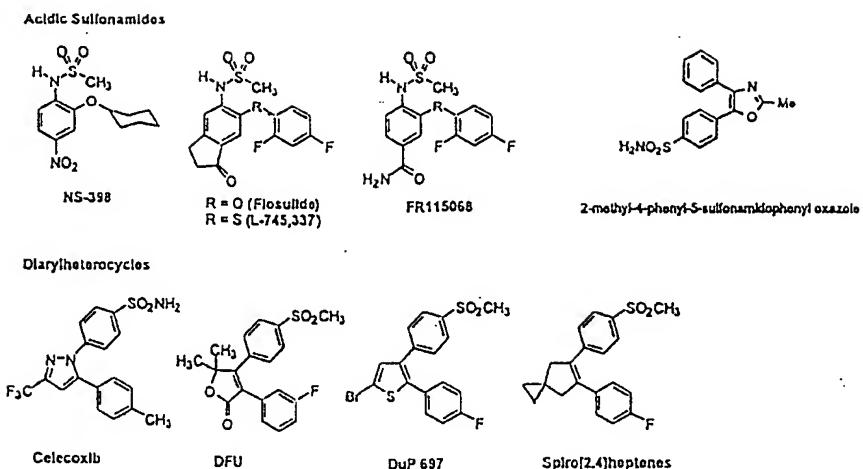
-10-

Antipyretic Effects, which Causes Minimal Stomach Lesions", *Gen. Pharmacol.* (1993) Vol. 24, pp. 105-110; Wiesenbergs-Boetcher, Schweizer, Green, Muller, Maerki, and Pfeilschifter, "The Pharmacological Profile of CGP 28238, A Novel Highly Potent Anti-Inflammatory Compound", *Drugs Exptl Clin Res.* (1989) Vol. XV, pp. 501-509; Futaki, Takahashi, Yokoyama, Arai, Higuchi, and Otomo, "NS-398, A New Anti-Inflammatory Agent, Selectively Inhibits Prostaglandin G/H Synthase/Cyclooxygenase (COX-2) Activity *in vitro*", *Prostaglandins* (1994) Vol. 47, pp. 55-59; Klein, Nusing, Pfeilschifter, and Ullrich, "Selective Inhibition of Cyclooxygenase-2", *Biochem. Pharmacol.* (1994) Vol. 48, pp. 1605-1610; Li, Black, Chan, Ford-Hutchinson, Gauthier, Gordon, Guay, Kargman, Lau, Mancini, Quimet, Roy, Vickers, Wong, Young, Zamboni, and Prasit, "Cyclooxygenase-2 Inhibitors. Synthesis and Pharmacological Activities of 5-Methanesulfonamido-1-indanone Derivatives", *J. Med. Chem.* (1995) Vol. 38, pp. 4897-8905; Prasit, Black, Chan, Ford-Hutchinson, Gauthier, Gordon, Guay, Kargman, Lau, Li, Mancini, Quimet, Roy, Tagari, Vickers, Wong, Young, and Zamboni, "L-745,337: A Selective Cyclooxygenase-2 Inhibitor", *Med. Chem. Res.* (1995) Vol. 5, pp. 364-374; Tanaka, Shimotori, Makino, Aikawa, Inaba, Yoshida, and Takano, "Pharmacological Studies of the New Antiinflammatory Agent 3-Formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one. 1st Communication: Antiinflammatory, Analgesic and Other Related Properties", *Arzniem.-Forsch./Drug Res.* (1992) Vol. 42, pp. 935-944; Nakamura, Tsuji, Konishi, Okumura, and Matsuo, "Studies on Anti-Inflammatory Agents. I. Synthesis and Pharmacological Properties of 2'-

-11-

(phenylthio)methanesulfonamides and Related Derivatives", *Chem. Pharm. Bull.* (1993) Vol. 41, pp. 894-906; Chan, Boyce, Brideau, Ford-Hutchinson, Gordon, Guay, Hill, Li, Mancini, Penneton, Prasit, Rasori, Riendeau, Roy, Tagari, Vickers, Wong, and Rodger, "Pharmacology of a Selective 5 Cyclooxygenase-2 Inhibitor, L-745,337: A Novel Nonsteroidal Anti-Inflammatory Agent with an Ulcerogenic Sparing Effect in Rat and Nonhuman Primate Stomach", *J. Pharmacol. Exp. Ther.* (1995) Vol. 274, pp. 1531-1537; and Graedon and Graedon, "Pills Promise Relief without Ulcers", *The Raleigh, North Carolina News and Observer*, p. 8D (September 13, 1998) which 10 addresses, in general terms, the development of celecoxib, meloxicam, and vioxx as selective COX-2 inhibitors.

Representative acidic sulfonamides and diarylheterocyclics that have been reported as selective COX-2 inhibitors in the journal articles mentioned in the above paragraph are:



Although acidic sulfonamides and diarylheterocyclics have been extensively studied as selective COX-2 inhibitors, there are very few reports on converting NSAIDs that are selective COX-1 inhibitors into selective COX-2 inhibitors. See, Black, Bayly, Belley, Chan, Charleson, Denis, Gauthier,
5 Gordon, Guay, Kargman, Lau, Leblanc, Mancini, Quellet, Percival, Roy, Skorey, Tagari, Vickers, Wong, Xu, and Prasit, "From Indomethacin to a Selective COX-2 Inhibitor: Development of Indolalkanoic Acids as Potent and Selective Cyclooxygenase-2 Inhibitors", *Bioorg. Med. Chem. Lett.* (1996) Vol. 6, pp. 725-730; Luong, Miller, Barnett, Chow, Ramesha, and Browner,
10 "Flexibility of the NSAID Binding Site in the Structure of Human Cyclooxygenase-2", *Nature Structural Biol.* (1996) Vol. 3, pp. 927-933; and Kalgutkar, Crews, Rowlinson, Garner, Seibert, and Marnett, "Aspirin-Like

-13-

Molecules that Covalently Inactivate Cyclooxygenase-2", *Science* (1998) Vol. 280, pp. 1268-1270.

Also, interesting is U.S. Patent No. 5,681,964 (issued in 1997) to Ashton et al., assignors to the University of Kentucky Research Foundation, which 5 shows conversion of indomethacin (an NSAID) into certain ester derivatives with concomitant reduction of GI irritation (see, Figure 1 of U.S. Patent No. 5,681,964 for the structure of the ester derivatives). Additionally, U.S. Patent Nos. 5,607,966 (Parent) (issued in 1997) and 5,811,438 (CIP) (issued in 1998), both to Hellberg et al., assignors to Alcon Laboratories, show conversion of 10 various NSAIDs (such as indomethacin) into certain ester derivatives and amide derivatives (which are useful as antioxidants and inhibitors of 5-lipoxygenase) but do not address COX-2 selective inhibition.

Moreover, although U.S. Patent Nos. 3,285,908 (issued in 1966) and 3,336,194 (issued in 1967), both to Shen, assignor to Merck & Co., Inc., 15 describe various secondary and tertiary amide derivatives of indomethacin, the patents fail to address COX inhibition, probably because COX inhibition (both COX-1 and COX-2) was undiscovered in the 1960's, and thus fail to recognize that tertiary amide derivatives do not inhibit either COX-1 or COX-2. (Also, see comparison compounds 9 and 10 in the Examples below.) However, U.S. 20 Patent Nos. 5,436,265 (issued in 1995) to Black et al. and 5,510,368 (issued in 1996) to Lau et al., both patents assigned to Merck Frosst Canada, Inc., describe, respectively, 1-aryl-3-indolyl alkanoic acids and N-benzyl-3-indoleacetic acids as COX-2 selective inhibitors.

-14-

In the present investigation, the possibility has been explored for designing selective COX-2 inhibitors using as templates various compounds, such as NSAIDs, (1) that are selective COX-1 inhibitors or (2) that have essentially the same inhibitory activity for both COX-1 and COX-2. These two 5 kinds of compounds are collectively referred to as compounds that are not selective COX-2 inhibitors.

More particularly, analysis of the human COX-2 crystal structure complexed with zomepirac-derived selective COX-2 inhibitors indicates that the structural basis for selectivity by zomepirac-derived compounds is different 10 from that of diarylheterocyclics. See, Luong et al. mentioned above. Unlike diarylheterocyclics, zomepirac analogs do not utilize the side pocket; instead they breach the constriction at the mouth of the COX active site occupied by Arg106 and Tyr341 and project down the lobby region. The projection into this 15 sterically uncongested region in the COX-2 active site opens the possibility that making a wide range of analogs of COOH-containing NSAIDs, each with a different pendent functional group replacing the OH of the COOH, would accomplish many purposes related to drug discovery or development. For example, certain pendent groups could improve water-solubility, bioavailability, or pharmacokinetics. Another possibility would be to attach a pendent 20 pharmacophore in order to target a completely different protein leading to compounds with dual pharmacological functions.

Abbott Laboratories and Parke-Davis have attempted the pharmacophore approach. See, respectively, Kolasa, Brooks, Rodrigues,

-15-

Summers, Dellaria, Hulkower, Bouska, Bell, and Carter, "Nonsteroidal Anti-Inflammatory Drugs as Scaffolds for the Design of 5-Lipoxygenase Inhibitors", *J. Med. Chem.* (1997) Vol. 40, pp. 819-824; and Flynn, Capiris, Cetenko, Connor, Dyer, Kostlan, Niese, Schrier, and Sircar, "Nonsteroidal 5 Antiinflammatory Drug Hydroxamic Acids. Dual Inhibitors of Both Cyclooxygenase and 5-Lipoxygenase", *J. Med. Chem.* (1990) Vol. 33, pp. 2070-2072. Both Kolasa et al. and Flynn et al. reported that replacement of the carboxylic acid group in NSAIDs with a hydroxamic acid moiety or a hydroxyurea moiety provided dual inhibitors of COX and 5-lipoxygenase.

Nevertheless, none of the analogs displayed any significant selective COX-2 inhibition, and furthermore the hydroxamates underwent facile hydrolysis.

However, nothing in the above-discussed literature suggests that converting a COOH-containing drug, such as a COOH-containing NSAID, that is not selective for COX-2 inhibition into a derivative that is selective for COX-2 inhibition would also result in that derivative being a cancer inhibitor. Nevertheless, it is interesting to note that sulindac sulfide (an NSAID which contains a COOH moiety as well as a methyl sulfide moiety) is a 40-fold more potent inhibitor against COX-1 than COX-2, yet also exhibits inhibition of tumors. On the other hand, a derivative, namely sulindac sulfone (which contains a COOH moiety as well as a methyl sulfone moiety) does not inhibit either COX-1 or COX-2, but still exhibits inhibition of tumors.

Thus, it would be desirable to find certain COOH-containing drugs, such as COOH-containing NSAIDs, which are not selective COX-2 inhibitors (either

-16-

display an inhibition for COX-1 far exceeding inhibition of COX-2 or display
essentially the same inhibition for COX-1 and COX-2) that would, when
converted into certain derivatives, become selective COX-2 inhibitors (display
an inhibition for COX-2 far exceeding inhibition for COX-1), as well as retain the
5 analgesic, antiinflammatory, and/or antipyretic effect of the drug, and yet exhibit
other effects not exhibited by the drug prior to derivatization, such as cancer
inhibition.

Summary and Objects of the Invention

Surprisingly with the present invention, it has been found that
10 derivatization of the carboxylic acid moiety of certain compounds, such as
certain NSAIDs, that are not selective COX-2 inhibitors, such as indomethacin,
into secondary amide analogs creates isozyme specificity for COX-2.
Moreover, the resultant secondary amide derivative is not only a selective
COX-2 inhibitor, but also is a cancer inhibitor, i.e., exhibits antiangiogenic
15 and/or antitumorigenic activity, and preferably, also retains the analgesic,
antiinflammatory, and/or antipyretic of the compound.

Therefore, the present invention provides a method for cancer treatment
in a warm blooded vertebrate animal. The method comprises administering to
the animal a treatment effective amount sufficient to inhibit cancer of a
20 carboxylic acid secondary amide derivative of a compound. The derivative is
selective for inhibition of cyclooxygenase-2. The compound (a) is a
cyclooxygenase inhibitor but is not selective for inhibition of cyclooxygenase-2

-17-

and (b) contains a carboxylic acid moiety or a pharmaceutically acceptable salt thereof. Preferably, the compound is a non-steroidal antiinflammatory drug, or a pharmaceutically acceptable salt thereof.

Hence, it is an object of the invention to provide a cancer treatment that
5 minimizes or obviates GI irritation.

Moreover, it is an advantage of the present invention that the cancer treatment is also analgesic, antiinflammatory, and/or antipyretic, absent the concomitant administration of an analgesic, antiinflammatory, and/or antipyretic drug, such as an NSAID or a pharmaceutically acceptable salt thereof.

10 Some of the objects of the invention having been stated above, other objects will become evident as the description proceeds, when taken in connection with the Laboratory Examples as described below.

Detailed Description of the Invention

The present invention involves a method for treating cancer in an animal
15 that is a warm-blooded vertebrate. Therefore, the invention concerns mammals and birds.

Contemplated is the treatment of mammals such as humans, as well as those mammals of importance due to being endangered (such as Siberian tigers), of economical importance (animals raised on farms for consumption by
20 humans) and/or social importance (animals kept as pets or in zoos) to humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants (such as cattle, oxen, sheep, giraffes,

-18-

deer, goats, bison, and camels), and horses. Also contemplated is the treatment of birds, including the treatment of those kinds of birds that are endangered, kept in zoos, as well as fowl, and more particularly domesticated fowl, i.e., poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economical importance to humans.

Thus, contemplated is the treatment of livestock, including, but not limited to, domesticated swine (pigs and hogs), ruminants, horses, poultry, and the like.

More particularly, a treatment effective amount of a secondary amide derivative of a carboxylic acid-containing compound is administered to the warm-blooded vertebrate animal. Thus, the invention comprises administration of the secondary amide derivative in concentrations calculated to provide the animal being treated with the appropriate milieu to provide prevention, control, or cessation of cancer. Moreover, in the preferred embodiment, the secondary amide derivative possesses an analgesic, antiinflammatory, and/or antipyretic property as possessed by the carboxylic acid-containing compound prior to derivatization, and thus, the cancer treatment provides an analgesic, antiinflammatory, and/or antipyretic effect in the animal and is free of concomitant administration of another drug for providing such effect.

By carboxylic-acid containing compound or COOH-containing compound as used herein in connection with the present invention, it is intended to include pharmaceutically acceptable acid salts of the compound. Thus, for instance, the COOH moiety includes COOM, where M is Na and the like.

The derivatives useful in the method of the present invention are secondary amide derivatives of drugs having a carboxylic acid moiety or a pharmaceutically acceptable salt thereof, for instance, secondary amide derivatives of non-steroidal anti-inflammatory drugs having a carboxylic acid moiety or a pharmaceutically acceptable salt thereof. A number of chemical classes of non-steroidal anti-inflammatory drugs have been identified, as described in *CRC Handbook of Eicosanoids: Prostaglandins, and Related Lipids, Volume II, Drugs Acting Via the Eicosanoids*, pages 59-133, CRC Press, Boca Raton, Fla. (1989).

10 The NSAID may be selected, therefore, from a variety of chemical classes including, but not limited to, fenamic acids, such as flufenamic acid, niflumic acid, and mefenamic acid; indoles, such as indomethacin, sulindac, and tolmetin; phenylalkanoic acids, such as suprofen, ketorolac, flurbiprofen, and ibuprofen; and phenylacetic acids, such as diclofenac. Further examples
15 of NSAIDs are listed below:

acecloferac	etodolac acid	loxoprofen
alcofenac	fenbufen	meclofenamate
amfenac	fenclofenac	naproxen
benoxaprofen	fencloxac	orpanoxin
20 bromfenac	fenoprofen	pirprofen
carprofen	flecloxic acid	pranoprofen
clidanac	indoprofen	tolfenamic acid
diflunisal	isofezolac	zaltoprofen
efenamic acid	ketoprofen	zomopirac

-20-

More specifically, preferred secondary amide derivatives useful in the present invention include, but are not limited to, secondary amide derivatives of the following COOH-containing NSAIDs: 6-methoxy- α -methyl-2-naphthylacetic acid (and its Na acid salt form known as naproxen), meclofenamic acid, and diclofenac, with secondary amide derivatives of indomethacin being preferred, and that indomethacin derivative described below as compound 11 being especially preferred. Also, the secondary amide derivatives of indomethacin, where the Cl at the 4-position of the benzoyl moiety is replaced with Br or F, should work in the present invention.

Even more preferred are the secondary amide derivatives of indomethacin including, but are not limited to, indomethacin-*N*-methyl amide, indomethacin-*N*-ethan-2-ol amide, indomethacin-*N*-octyl amide, indomethacin-*N*-nonyl amide, indomethacin-*N*-(2-methylbenzyl) amide, indomethacin-*N*-(4-methylbenzyl) amide, indomethacin-*N*-((R)-4-dimethylbenzyl) amide, indomethacin-*N*-(S)-4-dimethylbenzyl) amide, indomethacin-*N*-(2-phenethyl) amide, indomethacin-*N*-(4-fluorophenyl) amide, indomethacin-*N*-(4-chlorophenyl) amide, indomethacin-*N*-(4-acetamidophenyl) amide, indomethacin-*N*-(4-methylmercapto)phenyl amide, indomethacin-*N*-(3-methylmercaptophenyl) amide, indomethacin-*N*-(4-methoxyphenyl) amide, indomethacin-*N*-(3-ethoxyphenyl) amide, indomethacin-*N*-(3,4,5-trimethoxyphenyl) amide, indomethacin-*N*-(3-pyridyl) amide, indomethacin-*N*-(2-chloro)pyridyl) amide, indomethacin-*N*-5-((1-ethyl)pyrazolo) amide, indomethacin-*N*-(3-chloropropyl) amide, indomethacin-*N*-

-21-

methoxycarbonylmethyl amide, indomethacin-N-2-(2-L-methoxycarbonylethyl) amide, indomethacin-N-2-(2-D-methoxycarbonylethyl) amide, indomethacin-N-(4-methoxycarbonylbenzyl) amide, indomethacin-N-(4-methoxycarbonylmethylphenyl) amide, indomethacin-N-(2-pyrazinyl) amide,
5 indomethacin-N-2-(4-methylthiazolyl) amide, indomethacin-N-(4-biphenyl) amide, and combinations thereof.

The secondary amide derivative may be administered to the animal as a suppository or as a supplement to fluids that are administered internally or parenterally, for instance nutrient fluids such as intravenous sucrose
10 solutions. Furthermore, intraoral (such as buccal or sublingual) administration or transdermal (such as with a skin patch) administration to the animal is also contemplated. A good discussion of intraoral administration can be seen in U.S. Patent No. 4,229,447 issued October 21, 1980 to Porter and U.S. Patent No. 5,504,086 issued April 2, 1996 to Ellinwood and Gupta. A good discussion
15 of transdermal administration can be seen in U.S. Patent No. 5,016,652 issued May 21, 1991 to Rose and Jarvik.

Additionally, administration to the animal may be by various oral methods, for instance as a tablet, capsule, or powder (crystalline form) that is swallowed. Also, oral administration may include that the secondary amide
20 derivative is admixed in a carrier fluid appropriate therefor so that it is administered as a liquid (solution or suspension) that is drunk. When the secondary amide derivative is admixed in a carrier fluid, appropriate fluids include, but are not limited to, water, rehydration solutions (i.e., water with

-22-

electrolytes such as potassium citrate and sodium chloride, for instance the solution available under the trade name RESOL® from Wyeth Laboratories), nutritional fluids (i.e., milk, fruit juice), and combinations thereof. Thus, the oral administration may be as a component of the diet, such as human food, animal feed, and combinations thereof.

In addition to oral administration such as by way of the mouth, contemplated also is administration of a solution or suspension to the esophagus, stomach, and/or duodenum, such as by gavage, i.e., by way of a feeding tube. Gavage type of administration is useful for when the cancer has progressed and the animal can no longer swallow food, medicine, et cetera, by mouth.

Hence, it is also contemplated that additional ingredients, such as various excipients, carriers, surfactants, nutriments, and the like, as well as various medicaments other than a secondary amide derivative, or combinations thereof, may be present together with the secondary amide derivative, whatever the form that the derivative is in. Medicaments other than a secondary amide derivative may include, but are not limited to, osmolytic polyols and osmolytic amino acids (i.e., myo-inositol, sorbitol, glycine, alanine, glutamine, glutamate, aspartate, proline, and taurine), cardiotonics (i.e., glycocyamine), analgesics, antibiotics, electrolytes (i.e., organic or mineral electrolytes such as salts), and combinations thereof.

A suitable dosing amount of secondary amide derivative for administration to the animal should range from about 0.5 mg to about 7.0 mg

-23-

per kg of body weight of the animal per day, more preferably from about 1.5 mg to about 6.0 mg per kg of body weight of the animal per day, and even more preferably from about 2.0 mg to about 5.0 mg per kg of body weight of the animal per day. Administration may be one or more times per day to achieve

5 the total desired daily dose. Of course, the amount can vary depending on the severity of the cancer and/or the age of the animal.

The present invention should be useful in the treatment of cancer in animals, wherein the cancer is caused by pathogens (i.e., parasites, bacteria, protozoa, and viruses, including toxic agents in food), nutritional factors (i.e.,

10 excess mineral salts, excess protein, allergic agents in food, undigestible food components, or poor quality ingredients in food), environmental factors that act as stressors or pollutants (i.e., heat, chilling, shipment of animals, or toxins such as from air and/or water pollution), and/or physiological disorders such as those of the digestive tract, pulmonary/circulatory system, liver, kidneys, colon,

15 and/or pancreas.

The present invention indicates that COOH-containing drugs that are not COX-2 selective inhibitors, such as the NSAID known as indomethacin, when converted into secondary amides, results in isozyme specificity for COX-2 and thus presents an efficient strategy for the generation of potent and selective

20 COX-2 inhibitors. The below-discussed extensive SAR study conducted with indomethacin suggests that a variety of secondary amide substituents are tolerated for replacing the OH in the COOH moiety of indomethacin, and these resultant derivatives are as potent and selective as COX-2 inhibitors as are the

-24-

diarylheterocyclics discussed above. Thus, this strategy has great potential in the development of nonulcerogenic antiinflammatory agents. Moreover, as illustrated in the Examples below, the secondary amide derivatives of the invention also exhibit an anticancer effect.

5

Laboratory Examples

The following is noted in connection with the materials and procedures below.

The amides that were made and their selective COX-2 inhibition properties are listed in the Table below. A total of 31 analogs (31 amide 10 derivatives) of indomethacin were prepared. Various nitrogen-containing substituents (i.e., amines) that replaced the OH of the COOH, in order to create an amide, included aminoalkyl, aminoaryl, aminoarylalkyl, aminoethers, or aminopyridinyl moieties as part of the nitrogen-containing substituent. The most potent amide analogs in the indomethacin derivative series displayed IC₅₀ 15 values for inhibition of purified human COX-2 in the low nanomolar range with COX-2 selectivity ratios ranging from > 1000 to 4000.

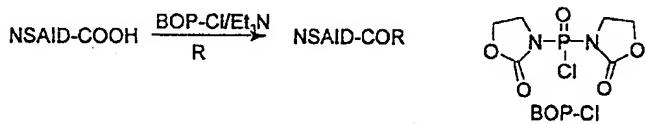
Well established methodology was utilized in the synthesis of amide derivatives of indomethacin by treatment of the indomethacin with an appropriate amine (designated as R) utilizing BOP-Cl as the carboxylic acid 20 activator to replace the OH of the COOH with R and create an amide. If R was a primary amine, the resultant derivative was a secondary amide, and if R was a secondary amine, the resultant derivative was a tertiary amide.

-25-

More specifically, a reaction mixture containing indomethacin (300 mg, 0.84 mmol) and BOP-Cl (218 mg, 0.84 mmol) in 5 mL of anhydrous CH₂Cl₂ was treated with Et₃N (167 mg, 0.84 mmol) and allowed to stir at rt for 10 minutes. The mixture was then treated with the appropriate amine (0.94 mmol)

5 designated as R and stirred overnight at rt. Following dilution with CH₂Cl₂ (30 mL), the resultant solution was washed with water (2 x 25 mL), 3 N NaOH (2 x 25 mL), water (2 x 30 mL), dried (in the presence of MgSO₄), filtered, and the solvent concentrated in vacuo. The crude amide was purified by chromatography on silica gel or recrystallization in the appropriate solvent.

10 The reaction scheme was as follows:



IC₅₀ values for the inhibition of purified human COX-2 or ovine COX-1 by test compounds were determined by the TLC assay discussed below. Ovine COX-1 was used because it is very easy to isolate and to purify the enzyme from sheep seminal vesicles, whereas human COX-1 is normally obtained by over expression in an insect cell system and is very difficult to handle and especially to purify. COX-1 from sheep is >90% similar to COX-1 from humans. Finally, inhibition of COX-1 from these two sources by NSAIDs has been reported in the published literature and the IC₅₀ values are similar,

-26-

suggesting no dramatic active site differences. COX-1 was purified from ram seminal vesicles obtained from Oxford Biomedical Research, Inc. (Oxford, Michigan). The specific activity of the protein was 20 ($\mu\text{M}\text{O}_2/\text{minute}/\text{mg}$), and the percentage of holoprotein was 13.5%. Samples of human COX-2 (1.62
5 $\mu\text{g}/\mu\text{l}$) were obtained by expression of insect cell cloned human COX-2 carried on baculovirus vectors, followed by purification.

The enzymes obtained after purification were apo (i.e., they lacked the hemprosthetic group). They were reconstituted with hemein purchased from Sigma Chemical Co. (St. Louis, Missouri) in the assays to render them to their
10 natural states which is holo (i.e., natural COX-1 and natural COX-2 contain the hemprosthetic group) so that the inhibition by test compounds had physiological relevance.

HoloCOX-2 (66 nM) or holoCOX-1 (44 nM) in 100 mM Tris-HCl, pH 8.0 containing 500 μM phenol was treated with several concentrations of
15 indomethacin or an amide derivative of indomethacin at 25 °C for 20 minutes. Since the recombinant COX-2 had a lower specific activity than the ovine COX-1, the protein concentrations were adjusted such that the percentages of total products obtained following catalysis of arachidonic acid (purchased from Nu Chek Prep, Elysian, Minnesota) by the two isoforms were comparable.

20 More specifically, time- and concentration-dependent inhibition of cyclooxygenase activity for ovine COX-1 (44 nM) and human COX-2 (66 nM) using the TLC assay was determined as follows. Reaction mixtures of 200 μL contained hemein-reconstituted protein in 100 mM Tris-HCl, pH 8.0, 500 μM

-27-

phenol, and [1-¹⁴C]-arachidonic acid (50 µM, ~ 55-57 mCi/mmol). For the time-dependent inhibition assay, hematin-reconstituted COX-1 (44 nM) or COX-2 (66 nM) was preincubated at rt for 20 minutes with varying inhibitor concentrations in DMSO followed by the addition of [1-¹⁴C]-arachidonic acid (50 µM) for 30 seconds at 37 °C. [1-¹⁴C]-arachidonic acid (~55-57 mCi/mmol) was purchased from New England Nuclear, Dupont, or American Radiolabeled Chemicals (St. Louis, Missouri).

Reactions were terminated by solvent extraction in Et₂O/CH₃OH/1 M citrate, pH 4.0 (30:4:1). The phases were separated by centrifugation at 2000 g-force for 2 minutes and the organic phase was spotted on a TLC plate (obtained from J. T. Baker, Phillipsburg, New Jersey). The plate was developed in EtOAc/CH₂Cl₂/glacial AcOH (75:25:1) at 4 °C. Radiolabeled prostanoid products were quantitatively determined with a radioactivity scanner (obtained from Bioscan, Inc., Washington, D.C.). The percentage of total products observed at different inhibitor concentrations was divided by the percentage of products observed for protein samples preincubated for the same time with DMSO.

Control experiments in the absence of indomethacin indicated ~25-30% conversion of fatty acid substrate to products, which was sufficient for assessing the inhibitory properties of all test compounds. Under these conditions, indomethacin displayed selective time- and concentration-dependent inhibition of COX-1 (i.e., IC₅₀ (COX-1) ~ 0.050 µM; IC₅₀ (COX-2) ~ 0.75 µM), whereas the secondary amide derivatives displayed selective COX-2

-28-

inhibition and the tertiary amide derivatives did not inhibit either COX-1 or COX-2 (i.e., measurement of COX-2 was stopped at an extremely high IC₅₀ and still >80% COX-2 activity remained). Also, the following is noted for NS-398 and 2-methyl-4-phenyl-5-sulfoamidophenyl oxazole, which are two of the above-5 mentioned acidic sulfonamides: namely, NS-398: IC₅₀ (COX-2) ~ 0.12 µM; IC₅₀ (COX-1) > 66 µM; and 2-methyl-4-phenyl-5-sulfoamidophenyl oxazole: IC₅₀ (COX-2) ~ 0.06 µM; IC₅₀ (COX-1) > 66 µM.

For certain comparison tests, inhibition of COX-2 activity in activated murine RAW264.7 cells was determined as follows. Low passage number 10 murine RAW264.7 cells were grown in DMEM containing 10% heat-inactivated FBS. Cells (6.2×10^6 cells / T25 flask) were activated with 500 ng/mL LPS and 10 units/mL IFN-g in serum-free DMEM for 7 hours. Vehicle (DMSO) or inhibitor in DMSO (0 to 1 µM) was added for 30 minutes at 37 °C. Inhibition of exogenous arachidonic acid metabolism or inhibition of PGD₂ synthesis was 15 determined by incubating the respective cells with 20 µM ¹⁴C-AA for 15 minutes at 25 °C. Aliquots (200 µL) were removed into termination solution and total products were quantitatively determined by the TLC assay as described earlier.

Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. Chemical yields were unoptimized specific 20 examples of one preparation. NSAIDs (i.e., indomethacin) were purchased from Sigma (St. Louis, Missouri). All other chemicals were purchased from Aldrich (Milwaukee, Wisconsin). Methylene chloride was purchased as anhydrous from Aldrich and was used as received. All other solvents were

-29-

HPLC grade. Analytical TLC (Analtech uniplatesTM) was used to follow the course of reactions. Silica gel (Fisher, 60-100 mesh) was used for column chromatography. ¹H NMR and ¹³C NMR spectra in CDCl₃ were recorded on a Bruker WP-360 spectrometer or an AM-400 spectrometer. Chemical shifts 5 were expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard. Spin multiplicities were reported as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) were reported in hertz (Hz).

Example I

10 The following carboxylic acid amide derivatives of indomethacin, designated as compounds 1 through 31, were made. (Note: compounds 1, 2, and 9 through 13 are also disclosed in the above-discussed U.S. Patent Nos. 3,285,908 and 3,336,194, both to Shen, assignor to Merck & Co., Inc.)

Indomethacin-*N*-methyl amide (compound 1) was obtained upon 15 chromatography on silica gel (EtOAc:hexanes; 10:90 then 50:50) as a bright yellow solid (271 mg, 79%). mp = 187-189 °C; ¹H NMR (CDCl₃) δ 7.64-7.67 (dd, 2 H, J = 6.6 Hz and 1.9 Hz, ArH), 7.47-7.50 (dd, 2 H, J = 6.7 Hz and 1.9 Hz, ArH), 6.88-6.89 (dd, 1 H, J = 9.1 Hz and 2.5 Hz, ArH), 6.84-6.87 (d, 1 H, J = 9.0 Hz, ArH), 6.68-6.72 (dd, 1 H, J = 9.1 Hz and 2.5 Hz, ArH), 5.22 (bs, 1 H, NH), 3.83 (s, 3 H, CH₃), 3.65 (s, 2 H, CH₂), 2.75-2.76 (d, 3 H, J = 4.8 Hz, CH₃), 2.39 (s, 3 H, CH₃).

-30-

Indomethacin-*N*-ethan-2-ol amide (**compound 2**) was obtained upon chromatography on silica gel (EtOAc) as a pale yellow solid (143 mg, 39%).
mp = 162-164 °C; ¹H NMR (CDCl₃) δ 7.66-7.68 (dd, 2 H, J = 6.7 Hz and 1.7 Hz, ArH), 7.47-7.50 (dd, 2 H, J = 6.9 Hz and 1.9 Hz, ArH), 6.85-6.89 (d and s, 5 2 H, J = 9.2 Hz, ArH), 6.68-6.72 (dd, 1 H, J = 9.0 Hz and 2.5 Hz, ArH), 6.03 (bs, 1 H, NH), 3.82 (s, 3 H, CH₃), 3.67 (bs, 4 H, 2CH₂), 3.35-3.40 (q, 2 H, J = 4.8 Hz, CH₂), 2.44 (bs, 1 H, OH), 2.39 (s, 3 H, CH₃).

Indomethacin-*N*-octyl amide (**compound 3**) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70) as a yellow solid (164 mg, 42%). mp = 109-111 °C; ¹H NMR (CDCl₃) δ 7.62-7.65 (d, 2H, J= 8.2 Hz, ArH), 7.46-7.49 (d, 2H, J= 8.2 Hz, ArH), 6.85-6.89 (m, 2H, ArH), 6.68-6.71 (d, 1H, J= 8.9 Hz, ArH), 5.67 (s, 1H, NH), 3.82 (s, 3H, CH₃), 3.64 (s, 2H, CH₂), 3.16-3.22 (m, 2H, CH₂), 2.38 (s, 3H, CH₃), 1.39 (m, 2H, CH₂), 1.19 (m, 10 H, 10 5CH₂), 0.83-0.88 (t, J= 6.2 Hz, CH₃).

Indomethacin-*N*-nonyl amide (**compound 4**) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70) as a yellow solid (191 mg, 47%). mp = 128-130 °C; ¹H NMR (CDCl₃) δ 7.64-7.67 (d, 2H, J= 8.4 Hz, ArH), 7.47-7.50 (d, 2H, J= 8.4 Hz, ArH), 6.89 (s, 1H, ArH), 6.85-6.88 (d, J= 8.9 Hz, ArH), 6.68-6.72 (dd, 1H, J= 9.0 Hz and 2.4 Hz, ArH), 5.60-5.63 (bt, J= 5.3 Hz, NH), 3.82 (s, 3H, CH₃), 3.64 (s, 2H, CH₂), 3.16-3.22 (m, 2H, CH₂), 2.38 (s, 3H, CH₃), 1.36-1.41 (m, 2H, CH₂), 1.19-1.28 (m, 12H, 6CH₂), 0.84-0.89 (t, J= 6.5 Hz, CH₃).

-31-

Indomethacin-*N*-(2-methylbenzyl) amide (**compound 5**) was obtained upon chromatography on silica gel (EtOAc:hexanes; 50:50) as a yellow solid (218 mg, 56%). mp = 177-179 °C; ¹H NMR (CDCl₃) δ 7.60-7.61 (d, 2H, J= 8.1 Hz, ArH), 7.44-7.46 (d, 2H, J= 8.1 Hz, ArH), 7.06-7.15 (m, 4H, ArH), 6.83-6.89 (m, 2H, ArH), 6.67-6.70 (d, 1H, J= 8.1 Hz, ArH), 5.84 (s, 1H, NH), 4.40-4.41 (d, 2H, J= 5.3 Hz, CH₂), 3.79 (s, 3H, CH₃), 3.70 (s, 2H, CH₂), 2.37 (s, 3H, CH₃), 2.19 (s, 3H, CH₃).

Indomethacin-*N*-(4-methylbenzyl) amide (**compound 6**) was obtained upon recrystallization from methanol as a yellow solid (142 mg, 37%). mp = 191-192 °C; ¹H NMR (CDCl₃) δ 7.63-7.60 (d, 2H, J= 8.5 Hz, ArH), 7.46-7.44 (d, 2H, J= 8.4 Hz, ArH), 7.08-7.01 (m, 4H, ArH), 6.88 (s, 1H, ArH), 6.87-6.85 (d, 1H, J= 6.3 Hz, ArH), 6.71-6.67 (dd, 1H, J= 9.0 Hz and 2.4 Hz, ArH), 5.89 (bt, 1H, NH), 4.38-4.36 (d, 2H, J= 5.9 Hz, CH₂), 3.78 (s, 3H, CH₃), 3.69 (s, 2H, CH₂), 2.35 (s, 3H, CH₃), 2.30 (s, 3H, CH₃).

Indomethacin-*N*-((R)-4-dimethylbenzyl) amide (**compound 7**) was obtained upon recrystallization from methanol to yield a pale yellow solid (124 mg, 31%). mp = 201-202 °C; ¹H NMR (CDCl₃) δ 7.62-7.64 (d, 2H, J= 8.4 Hz, ArH), 7.45-7.48 (d, 2H, J= 8.6 Hz, ArH), 7.01-7.08 (m, 4H, ArH), 6.87-6.90 (d, 1H, J= 9.0 Hz, ArH), 6.83-6.84 (d, 1H, J= 2.3 Hz, ArH), 6.68-6.72 (dd, 1H, J= 9.0 Hz and 2.4 Hz, ArH), 5.76-5.78 (bd, 1H, J= 8.0 Hz, NH), 5.09-5.14 (m, 1H, CH), 3.76 (s, 3H, CH₃), 3.63-3.64 (d, 2H, J= 2.8 Hz, CH₂), 2.34 (s, 3H, CH₃), 2.30 (s, 3H, CH₃), 1.35-1.38 (d, 3H, J= 6.8 Hz, CH₃).

-32-

Indomethacin-*N*-(*S*)-4-dimethylbenzyl amide (**compound 8**) was obtained upon recrystallization from methanol as a pale yellow solid (163 mg, 41%). mp = 200-201 °C; ¹H NMR (CDCl₃) δ 7.53-7.55 (d, 2H, J= 8.3 Hz, ArH), 7.37-7.40 (d, 2H, J= 8.4 Hz, ArH), 6.94-7.01 (m, 4H, ArH), 6.76-6.82 (m, 2H, 5 ArH), 6.61-6.64 (dd, 1H, J= 9.0 Hz and 2.5 Hz, ArH) 5.77-5.79 (bd, 1H, J= 7.8 Hz, NH), 5.02-5.07 (m, 1H, CH), 3.69 (s, 3H, CH₃), 3.58-3.59 (d, 2H, J= 2.9 Hz, CH₂), 2.27 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 1.28-1.30 (d, 3H, J= 6.9 Hz, CH₃).

Comparison. Indomethacin-*N*-methylphenethyl amide (**compound 9**) was obtained upon chromatography on silica gel (EtOAc:hexanes; 50:50) as 10 a yellow solid (288 mg, 72%). mp = 61-63 °C; ¹H NMR (CDCl₃) δ 7.64-7.67 (d, 2H, J= 8.4 Hz, ArH), 7.45-7.48 (d, 2H, J= 8.5 Hz, ArH), 7.02 (d, 1H, J= 2.4 Hz, ArH), 6.81-6.84 (d, 1H, J= 9.0 Hz, ArH), 6.63-6.66 (dd, 1H, J= 9.0 Hz and 2.5 Hz, ArH), 3.82 (s, 3H, CH₃), 3.71 (s, 2H, CH₂), 3.57-3.60 (t, 2H, J= 5.4 Hz, CH₂), 3.43-3.46 (t, 2H, J= 5.3 Hz, CH₂), 2.38 (s, 3H, CH₃), 1.59-1.61 (m, 2H, 15 CH₂), 1.52-1.53 (m, 2H, CH₂), 1.42-1.43 (m, 2H, CH₂).

Comparison. Indomethacin-*N*-piperidinyl amide (**compound 10**) was obtained upon chromatography on silica gel (EtOAc:hexanes; 40:60) as a pale 20 yellow solid (146 mg, 41%). mp = 161-163 °C; ¹H NMR (CDCl₃) δ 7.64-7.67 (d, 2H, J= 8.4 Hz, ArH), 7.45-7.48 (d, 2H, J= 8.5 Hz, ArH), 7.02 (d, 1H, J= 2.4 Hz, ArH), 6.81-6.84 (d, 1H, J= 9.0 Hz, ArH), 6.63-6.66 (dd, 1H, J= 9.0 Hz and 2.5 Hz, ArH), 3.82 (s, 3H, CH₃), 3.71 (s, 2H, CH₂), 3.57-3.60 (t, 2H, J= 5.4 Hz,

-33-

CH₂), 3.43-3.46 (t, 2H, J= 5.3 Hz, CH₂), 2.38 (s, 3H, CH₃), 1.59-1.61 (m, 2H, CH₂), 1.52-1.53 (m, 2H, CH₂), 1.42-1.43 (m, 2H, CH₂).

Indomethacin-*N*-(2-phenethyl) amide (compound 11) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70) as a bright yellow solid (169 mg, 44%). mp = 148-150 °C; ¹H NMR (CDCl₃) δ 7.58-7.60 (d, J= 8.4 Hz, ArH), 7.46-7.48 (d, 2H, J= 8.5 Hz, ArH), 7.12-7.14 (m, 3H, ArH), 6.85-6.95 (m, 4H, ArH), 6.69-6.73 (dd, 1H, J= 8.9 Hz and 2.4 Hz, ArH), 5.61 (s, 1H, NH), 3.81 (s, 3H, CH₃), 3.59 (s, 2H, CH₂), 3.43-3.49 (m, 2H, CH₂), 2.68-2.72 (t, 2H, J= 6.7 Hz, CH₂), 2.04 (s, 3H, CH₃).

Indomethacin-*N*-(4-fluorophenyl) amide (compound 12) was obtained upon chromatography on silica gel (EtOAc:hexanes; 5:95 to 20:80) as an orange solid (217 mg, 57%). mp = 200-202 °C; ¹H NMR (CDCl₃) δ 7.65-7.67 (d, 2H, J= 8.3 Hz, ArH), 7.47-7.50 (d, 2H, J= 8.3 Hz, ArH), 7.32-7.35 (m, 3H, ArH), 6.94-6.99 (m, 3H, ArH, NH), 6.85-6.88 (d, 1H, J= 9.0 Hz, ArH), 6.70-6.73 (dd, 1H, J= 9.0 Hz and 2.0 Hz, ArH), 3.81 (s, 3H, CH₃), 3.79 (s, 2H, CH₂), 2.45 (s, 3H, CH₃).

Indomethacin-*N*-(4-chlorophenyl) amide (compound 13) was obtained upon recrystallization from methanol as a pale yellow solid (234 mg, 56%). mp = 209-210 °C; ¹H NMR (CDCl₃) δ 7.58-7.61 (d, 2H, J= 8.2 Hz, ArH), 7.40-7.42 (d, 2H, J= 8.2 Hz, ArH), 7.13-7.27 (m, 5H, ArH), 6.84 (s, 1H, NH), 6.77-6.80 (d, 1H, J= 9.0 Hz, ArH), 6.62-6.65 (d, 1H, J= 9.0 Hz, ArH), 3.72 (s, 2H, CH₂), 3.72 (s, 3H, CH₃), 2.37 (s, 3H, CH₃).

-34-

Indomethacin-N-(4-acetamidophenyl) amide (compound 14) was obtained upon recrystallization from methanol as a pale yellow solid (221 mg, 54%). mp = 256-257 °C; ¹H NMR (DMSO-d₆) δ 10.14 (s, 1H, NH), 9.86 (s, 1H, NH), 7.62-7.70 (m, 4H, ArH), 7.48 (s, 4H, ArH), 7.18 (d, 1H, J= 2.3 Hz, ArH), 6.90-6.93 (d, 1H, J= 9.0 Hz, ArH), 6.68-6.72 (dd, 1H, J= 9.1 Hz and 2.5 Hz, ArH), 3.73 (s, 3H, CH₃), 3.71 (s, 2H, CH₂), 2.27 (s, 3H, CH₃), 1.99 (s, 3H, CH₃).

Indomethacin-N-(4-methylmercapto)phenyl amide (compound 15) was obtained upon chromatography on silica gel (EtOAc:hexanes; 50:50) as a bright yellow solid (162 mg, 40%). mp = 195-196 °C; ¹H NMR (CDCl₃) δ 7.67-7.70 (d, 2 H, J = 8.4 Hz, ArH), 7.48-7.50 (d, 2 H, J = 8.4 Hz, ArH), 7.30-7.33 (d, 2 H, J = 8.6 Hz, ArH), 7.17-7.22 (m, 3 H, 2 ArH and NH), 6.92-6.93 (d, 1 H, J = 2.3 Hz, ArH), 6.85-6.88 (d, 1 H, J = 9.0 Hz, ArH), 6.69-6.73 (dd, 1 H, J = 9.0 Hz and 2.4 Hz, ArH), 3.80 (s, 3 H, CH₃), 3.79 (s, 2 H, CH₂), 2.45 (s, 3 H, C₃), 2.44 (s, 3 H, CH₃).

Indomethacin-N-(3-methylmercaptophenyl) amide (compound 16) was obtained upon chromatography on silica gel (EtOAc:hexanes; 15:85) as a yellow solid (218 mg, 54%). mp = 129-131 °C; ¹H NMR (CDCl₃) δ 7.62-7.64 (d, 2H, J= 8.2 Hz, ArH), 7.45-7.48 (d, 2H, J = 8.4 Hz, ArH), 7.39 (s, 1H, NH), 7.09-7.18 (m, 2H, ArH), 6.94-6.96 (m, 3H, ArH), 6.86-6.89 (d, 1H, J= 9.0 Hz), 6.69-6.72 (d, 1H, J= 8.9 Hz, ArH), 3.80 (s, 3H, CH₃), 3.78 (s, 2H, CH₂), 2.42 (s, 3H, CH₃).

-35-

Indomethacin-N-(4-methoxyphenyl) amide (compound 17) was obtained upon chromatography on silica gel (EtOAc:hexanes; 10:90 to 25:75) as an orange solid (239 mg, 61%). mp = 201-202 °C; ¹H NMR (CDCl₃) δ 7.67-7.70 (dd, 2H, J= 6.8 Hz and 1.8 Hz, ArH), 7.48-7.51 (d, 2H, J= 7.1 Hz, ArH), 5 7.28-7.29 (d, 1H, J= 2.0 Hz, ArH), 7.20 (s, 1H, NH), 6.94-6.95 (d, 1H, J= 2.4 Hz, ArH), 6.86-6.89 (d, 1H, J= 9.0 Hz, ArH), 6.78-6.84 (m, 2H, ArH), 6.69-6.73 (dd, 1H, J= 9.0 Hz and 2.4 Hz, ArH), 3.81 (s, 3H, CH₃), 3.79 (s, 2H, CH₂), 3.76 (s, 3H, CH₃), 2.45 (s, 3H, CH₃).

Indomethacin-N-(3-ethoxyphenyl) amide (compound 18) was 10 obtained upon recrystallization from methanol as a bright yellow solid (297 mg, 74%). mp = 152-154 °C; ¹H NMR (CDCl₃) δ 7.68-7.70 (d, 2H, J= 8.4 Hz, ArH), 7.48-7.51 (d, 2H, J= 8.4 Hz, ArH), 7.24 (s, 1H, NH), 7.13-7.18 (m, 2H, ArH), 6.94-6.82 (m, 3H, ArH), 6.70-6.73 (dd, 1H, J= 9.0 Hz and 2.4 Hz), 6.61-6.65 (dd, 1H, J= 8.2 Hz and 1.7 Hz, ArH), 3.96-4.03 (q, 2H, J = 7.0 Hz, CH₂), 3.81 15 (s, 3H, CH₃), 3.80 (s, 2H, CH₂), 2.45 (s, 3H, CH₃), 1.36-1.40 (t, 3H, J= 7.0 Hz, CH₃).

Indomethacin-N-(3,4,5-trimethoxyphenyl) amide (compound 19) was obtained upon chromatography on silica gel (EtOAc:hexanes; 10:90 to 30:70) as a light orange solid (191 mg, 44%). mp = 239-241 °C; ¹H NMR (CDCl₃) δ 20 7.67-7.69 (d, 2H, J=8.5 Hz, ArH), 7.48-7.51 (d, 2H, J= 8.5 Hz, ArH), 7.20 (s, 1H, NH), 6.94 (d, 1H, J= 8.9 Hz, ArH), 6.70-6.74 (m, 3H, ArH), 3.78-3.81 (m, 14H, 3CH₃ & CH₂), 2.45 (s, 3H, CH₃).

-36-

Indomethacin-*N*-(3-pyridyl) amide (compound 20) was obtained upon chromatography on silica gel (EtOAc:hexanes; 50:50 to 75:25) as a yellow solid (190 mg, 52%). mp = 204-205 °C; ¹H NMR (CDCl₃) δ 8.39-8.40 (d, 1H, J= 2.1 Hz, ArH), 8.32-8.34 (d, 1H, J= 4.4 Hz, ArH), 8.04-8.08 (m, 1H, ArH), 7.66-7.70 (m, 2H, ArH), 7.48-7.52 (m, 2H, ArH), 7.38 (s, 1H, NH), 7.22-7.25 (m, 1H, ArH), 6.93-6.94 (d, 1H, J= 2.4 Hz, ArH), 6.85-6.88 (d, 1H, J= 9.1 Hz, ArH), 6.70-6.74 (dd, 1H, J= 9.1 Hz and 2.5 Hz, ArH), 3.84 (s, 2H, CH₂), 3.81 (s, 3H, CH₃), 2.47 (s, 3H, CH₃).

Indomethacin-*N*-5-((2-chloro)pyridyl) amide (compound 21) was obtained upon chromatography on silica gel (EtOAc:hexanes; 5:95 to 50:50) as a pale yellow solid (221 mg, 56%). mp = 196-198 °C; ¹H NMR (CDCl₃) δ 8.19-8.20 (d, 1H, J= 2.8 Hz, ArH), 8.03-8.06 (dd, 1H, J= 8.7 Hz and 2.9 Hz, ArH), 7.59-7.63 (m, 2H, ArH), 7.46-7.51 (m, 3H, ArH), 7.24 (s, 1H, NH), 6.92-6.93 (d, 1H, J= 2.4 Hz, ArH), 6.84-6.87 (d, 1H, J= 9.0 Hz, ArH), 6.70-6.74 (dd, 1H, J= 9.1 Hz and 2.5 Hz, ArH), 3.84 (s, 2H, CH₂), 3.82 (s, 3H, CH₃), 2.46 (s, 3H, CH₃).

Indomethacin-*N*-5-((1-ethyl)pyrazolo) amide (compound 22) was obtained upon recrystallization from methanol as a pale yellow solid (153 mg, 40%). mp = 193-194 °C; ¹H NMR (CDCl₃) δ 7.99 (bs, 1H, NH), 7.66-7.68 (d, 2H, J= 8.2 Hz, ArH), 7.47-7.50 (m, 3H, ArH), 7.00 (s, 1H, ArH), 6.83-6.86 (d, 1H, J= 9.0 Hz, ArH), 6.69-6.72 (d, 1H, J= 8.9 Hz, ArH), 6.35 (s, 1H, ArH), 4.01-4.04 (bd, 2H, J= 6.8 Hz, CH₂), 3.90 (s, 2H, CH₂), 3.82 (s, 3H, CH₃), 2.47 (s, 3H, CH₃), 1.24-1.29 (t, 3H, J= 7.1 Hz, CH₃).

-37-

Indomethacin-*N*-(3-chloropropyl) amide (compound 23) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70) as a off-white solid (153 mg, 40%). ¹H NMR (DMSO-d₆) δ 8.11 (bs, 1H, NH), 7.62-7.69 (m, 4H, ArH), 7.09 (s, 1 H, ArH), 6.92-6.95 (d, 1 H, J = 8.9 Hz, ArH), 6.68-6.71 (d, 1 H, J = 8.8 Hz, ArH), 3.80 (s, 3H, CH₃), 3.58-3.67 (t, 2 H, J = 6.3 Hz, CH₂), 3.52 (s, 2H, CH₂), 3.15-3.17 (m, 2 H, CH₂), 2.20 (s, 3H, CH₃), 1.81-1.85 (t, 2 H, J= 6.5 Hz, CH₂).

Indomethacin-*N*-methoxycarbonylmethyl amide (compound 24) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70) as a yellow solid (265 mg, 76%). ¹H NMR (CDCl₃) δ 7.66-7.68 (dd, 2 H, J = 6.7 Hz and 1.7 Hz, ArH), 7.47-7.50 (dd, 2 H, J = 6.9 Hz and 1.9 Hz, ArH), 6.92-6.95 (m, 2 H, ArH), 6.70-6.73 (m, 1 H, ArH), 6.03 (bs, 1 H, NH), 3.98-4.00 (d, 2 H, J = 5.5 Hz, CH₂), 3.84 (s, 3 H, CH₃), 3.71 (s, 3 H, CH₃), 3.69 (s, 2 H, CH₂), 2.38 (s, 3 H, CH₃).

Indomethacin-*N*-2-(2-L-methoxycarbonylethyl) amide (compound 25) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70 and then 50:50) as a yellow solid (300 mg, 84%). ¹H NMR (CDCl₃) δ 7.67-7.70 (dd, 2 H, J = 8.5 Hz and 1.85 Hz, ArH), 7.47-7.50 (dd, 2 H, J = 8.4 Hz and 1.9 Hz, ArH), 6.91-6.96 (m, 2 H, ArH), 6.69-6.73 (m, 1 H, ArH), 6.16-6.18 (d, 1 H, J = 7.4 Hz, NH), 4.57-4.62 (m, 1 H, CH), 3.83 (s, 3 H, CH₃), 3.70 (s, 3 H, CH₃), 3.65 (s, 2 H, CH₂), 2.37 (s, 3 H, CH₃), 1.32-1.34 (d, 3 H, J = 7.2 Hz, CH₃).

Indomethacin-*N*-2-(2-D-methoxycarbonylethyl) amide (compound 26) was obtained upon chromatography on silica gel (EtOAc:hexanes; 40:60)

-38-

as a yellow solid (803 mg, 67%). ^1H NMR (CDCl_3) δ 7.67-7.70 (dd, 2 H, J = 8.5 Hz and 1.85 Hz, ArH), 7.47-7.50 (dd, 2 H, J = 8.4 Hz and 1.9 Hz, ArH), 6.91-6.96 (m, 2 H, ArH), 6.69-6.73 (dd, 1 H, ArH), 6.16-6.18 (d, 1 H, J = 7.4 Hz, NH), 4.57-4.62 (m, 1 H, CH), 3.83 (s, 3 H, CH_3), 3.70 (s, 3 H, CH_3), 3.65 (s, 2 H, CH_2), 2.36 (s, 3 H, CH_3), 1.32-1.34 (d, 3 H, J = 7.2 Hz, CH_3).
5

Indomethacin-*N*-(4-methoxycarbonylbenzyl) amide (compound 27)

was obtained upon chromatography on silica gel (EtOAc:hexanes; 40:60) as a yellow solid (198 mg, 47%). ^1H NMR (CDCl_3) δ 7.91-7.94 (d, 2 H, J = 6.8 Hz, ArH), 7.61-7.65 (d, 2H, J = 8.7 Hz, ArH), 7.45-7.48 (d, 2H, J = 9.0 Hz, ArH),
10 7.19-7.21 (d, 2H, J = 8.3 Hz, ArH), 6.83-6.88 (m, 2H, ArH), 6.68-6.72 (dd, 1H, J = 9.0 Hz and 2.4 Hz, ArH), 5.97-5.99 (bt, 1H, J = 5.9 Hz, NH), 4.45-4.47 (d, 2H, J = 6.1 Hz, CH_2), 3.90 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 3.72 (s, 2H, CH_2), 2.38 (s, 3H, CH_3).

Indomethacin-*N*-(4-methoxycarbonylmethylphenyl) amide

15 (compound 28) was obtained upon chromatography on silica gel (EtOAc:hexanes; 20:80) as an yellow solid (100 mg, 23%). ^1H NMR (CDCl_3) δ 7.67-7.70 (d, 2H, J =8.5 Hz, ArH), 7.48-7.51 (d, 2H, J =8.5 Hz, ArH), 7.33-7.36 (d, 2H, J = 8.4 Hz, ArH), 7.18-7.23 (d and bs, 3 H, ArH and NH), 6.92-6.93 (d, 1H, J = 2.3 Hz, ArH), 6.85-6.88 (d, 1H, J = 9.0 Hz, ArH), 6.70-6.73 (dd, 1H, J = 9.0 Hz and 2.0 Hz, ArH), 3.81 (s, 5H, CH_2 and CH_3), 3.67 (s, 3H, CH_3), 3.56 (s, 3H, CH_2), 2.45 (s, 3H, CH_3).
20

Indomethacin-*N*-(2-pyrazinyl) amide (compound 29) was obtained

upon chromatography on silica gel (EtOAc:hexanes; 30:70 to 50:50) as a bright

-39-

yellow solid (251 mg, 69%). ^1H NMR (CDCl_3) δ 9.58 (d, 1 H, J = 1.4 Hz, ArH), 8.33-8.34 (d, 1 H, J = 2.5 Hz, ArH), 8.16-8.17 (m, 1 H, ArH), 7.86 (bs, 1 H, NH), 7.69-7.71 (d, 2H, J = 8.5 Hz, ArH), 7.49-7.51 (d, 2H, J = 8.5 Hz, ArH), 6.92-6.93 (d, 1H, J = 2.4 Hz, ArH), 6.84-6.87 (d, 1H, J = 8.9 Hz, ArH), 6.70-6.72 (dd, 1H, 5 J = 9.0 Hz and 2.5 Hz, ArH), 3.86 (s, 2H, CH_2), 3.81 (s, 3H, CH_3), 2.47 (s, 3H, CH_3).

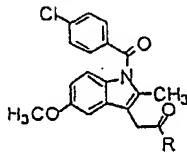
Indomethacin-*N*-2-(4-methylthiazolyl) amide (compound 30) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70 and then 70:30) to afford the pure product as a pale yellow solid which was recrystallized 10 from ethyl ether (241 mg, 63%). ^1H NMR (CDCl_3) δ 8.68 (bs, 1 H, NH), 7.70-7.74 (d, 2 H, J = 9.0 Hz, ArH), 7.48-7.52 (d, 2 H, J = 9.0 Hz, ArH), 6.79-6.85 (m, 2 H, ArH), 6.67-6.71 (dd, 1 H, J = 9.0 Hz and 2.4 Hz, ArH), 6.52 (s, 1 H, Thiazole-H), 3.88 (s, 2 H, CH_2), 3.79 (s, 3 H, CH_3), 2.45 (s, 3 H, CH_3), 2.27 (s, 3 H, CH_3).

15 Indomethacin-*N*-(4-biphenyl) amide (compound 31) was obtained upon chromatography on silica gel (EtOAc:hexanes; 30:70) to afford the pure product as a pale yellow solid (421 mg, 59%). ^1H NMR (CDCl_3) δ 7.68-7.71 (d, 2 H, J = 8.4 Hz, ArH), 7.32-7.55 (m, 11 H, ArH), 6.95-6.96 (d, 1 H, J = 2.0 Hz, ArH), 6.86-6.89 (d, 1 H, J = 9.0 Hz, ArH), 6.73-6.74 (dd, 1 H, J = 1.7 Hz, 20 ArH), 3.83 (s, 2 H, CH_2), 3.81 (s, 3 H, CH_3), 2.47 (s, 3 H, CH_3).

The structures and IC_{50} values for indomethacin and Compounds 1 through 31 are set out in the Table below.

-40-

Table Selective COX-2 Inhibition by Amide Derivatives of Indomethacin

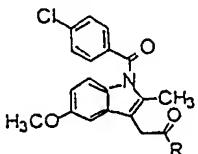


Compound	R	IC_{50} (μM) ^a		Selectivity ^b
		COX-2	COX-1	
Indomethacin	OH	0.75	0.05	0.066
1	NHCH ₃	0.70	> 66 ^c	> 94
2	HN~OH	0.25	> 66	287
3	HN~~~~~	0.0375	66	1760
4	HN~~~~~	0.04	16.5	412.5
5	HN~ CH ₃	0.15	> 66 ^c	> 440
6	HN~ CH ₃	0.06	8.0	133
7	HN~ CH ₃	0.0625	4.0	64
8	HN~ CH ₃	0.20	4.0	20
9 (Comparison)	N CH ₃	> 33 ^c	> 66	---
10 (Comparison)	N~	> 33 ^c	> 66	---
11	HN~	0.06	> 66	> 1100

^a IC_{50} values were determined by incubating several concn of inhibitor in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min followed by treatment with ^{14}C -AA (50 μM) at 37 °C for 30 sec. Assays were run in duplicate. ^b Ratio of IC_{50} (COX-1): IC_{50} (COX-2). ^c > 80% remaining COX-1 activity at this concn

-41-

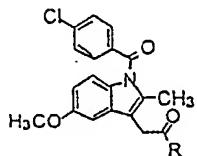
Table (Contd). Selective COX-2 Inhibition by Amide Derivatives of Indomethacin



Compound	R	IC_{50} (μM) ^a		Selectivity ^b
		COX-2	COX-1	
12		0.06	> 66 ^c	> 1100
13		0.062	> 66 ^c	> 1064
14		0.12	> 66 ^c	> 550
15		0.12	> 66 ^c	> 550
16		0.22	> 66 ^c	> 300
17		0.056	> 66 ^c	> 1178
18		0.65	52.5	81
19		> 1.0	> 66	> 66
20		0.052	> 66	> 1269
21		0.047	> 66 ^c	> 1404
22		0.70	> 66 ^c	> 94

-42-

Table (Contd). Selective COX-2 Inhibition by Amide Derivatives of Indomethacin



Compound	R	IC ₅₀ (μM) ^a		Selectivity ^b
		COX-2	COX-1	
23	HN-CH ₂ -Cl	0.050	45	900
24	HN-CH ₂ -C(=O)OCH ₃	4.0	> 66	> 16.5
25	HN-CH ₂ -C(=O)OCH ₃ CH ₃	0.4	> 66	> 165
26	HN-CH ₂ -C(=O)OCH ₃ CH ₃	0.19	> 66	> 347
27	HN--C(=O)OCH ₃	0.080	> 66	> 825
28	HN--CH ₂ -C(=O)OCH ₃	0.058	> 66	> 1138
29	HN-	4.0	> 66	> 16.5
30	HN-	4.0	> 66	> 16.5
31	HN-	0.5	> 66	> 132

^a IC₅₀ values were determined by incubating several concn of inhibitor in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min followed by treatment with 1-¹⁴C-AA (50 μM) at 37 °C for 30 sec. Assays were run in duplicate. ^b Ratio of IC₅₀ (COX-1):IC₅₀ (COX-2). ^c > 80% remaining COX-1 activity at this con

-43-

Discussion of Secondary Amide Derivatives of Indomethacin.

Carboxylic Acid Aliphatic Secondary Amides Derivatives of Indomethacin.

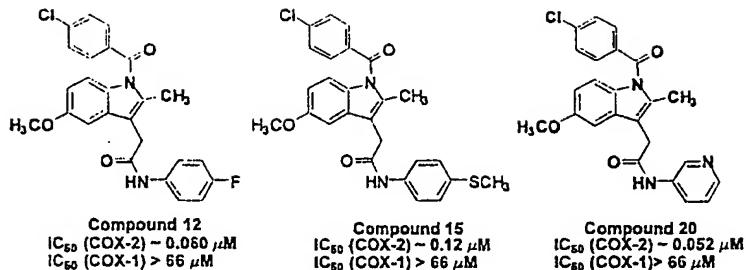
The N-methyl amide derivative (compound 1) displayed selective COX-2 inhibition (IC_{50} (COX-2) ~ 0.70 μ M; IC_{50} (COX-1) > 66 μ M). Increments in COX-
5 2 inhibitory potency and selectivity was observed with the higher octyl homolog
(compound 3); however, further increase in chain length to the nonyl derivative
(compound 4) led to some loss of COX-2 selectivity (compound 3: IC_{50} (COX-
2) ~ 37.5 nM; IC_{50} (COX-1) 66 μ M; compound 4: IC_{50} (COX-2) ~ 40 nM; IC_{50}
(COX-1) ~ 16.5 μ M).

10 **Carboxylic Acid Aromatic Secondary Amides Derivatives of Indomethacin.**

Incorporation of methylene spacer units (compound 11) between the amide nitrogen and the phenyl ring also generated potent and selective COX-2 inhibitors.

For instance, the 4-methylbenzyl amide derivative (compound 6) was
15 133-fold selective for COX-2, whereas the corresponding 2-methylbenzyl isomer (compound 5) was > 440 times more selective as a COX-2 inhibitor. Furthermore, the R--methyl-(4-methylbenzyl) enantiomer (compound 7) was a better inhibitor of COX-2 than the corresponding S-methyl enantiomer (compound 8).

20 Additionally, the aromatic amides containing the 4-fluoro (compound 12), 4-methylmercapto (compound 15), or the 3-pyridyl substituent (compound 20), displayed potent and selective COX-2 inhibition, as noted below.



Tertiary Amides (Comparison compounds 9 and 10).

Another interesting aspect in the SAR studies with the indomethacin amides was that *N,N*-methyl-2-phenethyl (compound 9) and the piperidinyl (compound 10) amide derivatives, both of which are tertiary amides, were inactive against COX-2. In other words, only the secondary amides were selective COX-2 inhibitors, whereas the tertiary amides were devoid of any inhibitory effect towards either isozyme i.e., measurement of COX-2 inhibition for the tertiary amides was stopped at an extremely high IC value (see the value of 33 for both compounds 9 and 10) and still >80% COX-1 activity remained.

Example II

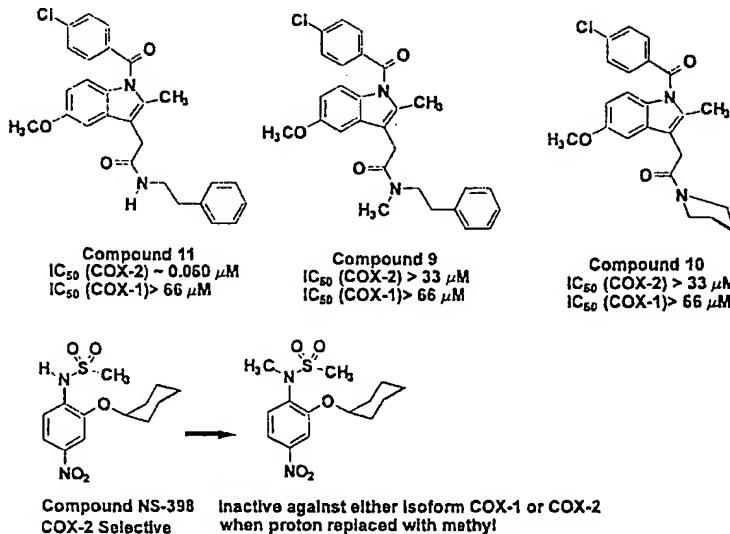
Comparison with Sulfonamides of Another Study.

A similar SAR study was previously reported in the above-noted journal article by Li et al. for acidic sulfonamides. (See, the structures drawn above for 5 compounds L-745,337 and NS-398.) Specifically, Li et al. found that replacement of the N-H proton in the NHSO_2CH_3 moiety of L-745,337 or NS-398 with a methyl group led to complete loss of inhibitory potency towards either the COX-1 or COX-2 isozyme.

This behaviour may be explained from the recently solved crystal 10 structure of murine COX-2 complexed with NS-398. See, Kurumbail et al., Abstract 197, Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases, *Fifth International Conference*, La Jolla, California (17-20 September 1997). Unlike the diarylheterocyclics, NS-398 does not utilize the side pocket even though it contains a sulfonamide group. Instead the 15 sulfonamide binds to Arg106 in a fashion similar to the carboxylic acid-containing NSAIDs.

Although the carboxylic acid secondary amide derivatives of indomethacin in the present invention do not contain any electron-withdrawing substituents, the above-discussed SAR observations on the lack of inhibition 20 by the carboxylic acid tertiary amide derivatives suggest that the -CONH-R₁ group probably binds to a group on the enzyme (see below). This can be seen from contrasting the data immediately below for the inventive secondary amide derivative (compound 11) with the comparison tertiary amide derivatives

(compounds 9 and 10) and the comparison derivative of prior art compound NS-398 in which the N-H proton in the NHSO_2CH_3 moiety was replaced with methyl.



Example III

5 Additional inhibitory activity testing with mouse COX.

Compound 11. The structural basis for COX-2 selectivity by compound 11 also was probed by site directed mutagenesis. More particularly, the inhibitory potency of indomethacin as compared to that of indomethacin-*N*-phenethyl amide (compound 11) was evaluated against site directed murine 10 COX-2 mutants (Arg106Gln and Tyr341Ala) which represent key residues involved in the binding of the carboxylic acid-containing NSAIDs. Arg106 is the only positively charged residue in the fatty acid binding site and is important for

binding the carboxylic acid moiety of an NSAID with Tyr341Ala, which is juxtaposed to Arg106 at the constriction site and is responsible for the selective binding of the *S*-enantiomers but not the *R*-enantiomers in the 2-phenylpropionate class of NSAIDs including flurbiprofen. In addition to these 5 mutants, also analyzed was the inhibition profile of the Val509IleArg499HisVal420Ile mutant (also known as the VRV mutant) which incorporates the major amino acid changes between COX-2 and COX-1 in the side pocket region and is responsible for binding the diarylheterocycles. The results were that indomethacin displayed a slightly better potency against wild-type mouse COX-2 than compound 11 (indomethacin: IC_{50} (mouse COX-2) ~ 25 nM; compound 11: IC_{50} (mouse COX-2) ~ 35 nM). Furthermore, the 10 Tyr341Ala and the triple mutant VRV was resistant to inhibition by each of indomethacin and compound 11, whereas the Arg106Gln mutant was resistant to inhibition by indomethacin but was effectively inhibited by compound 11 (IC_{50} 15 ~ 25 nM).

Compound 17. Inhibition of COX-2 activity in intact mouse cells by Compound 17 was assayed in murine RAW264.7 macrophages in which COX-2 activity was induced by pathologic stimuli. The macrophages were treated with LPS (500 ng/mL) and interferon- γ (10 U/mL) for 7.5 hours to induce COX-20 2 and then treated with several concentrations of the 4-methoxyphenyl amide derivative of indomethacin (compound 17) for 30 minutes at 37 °C. The IC_{50} value for PGD₂ by compound 17 was 62.5 nM. Under these conditions,

-48-

indomethacin was a better inhibitor of COX-2 activity in intact mouse cells (IC_{50} ~ 10 nM) than compound 17.

Indeed, comparison of the potency of indomethacin as a inhibitor of purified mouse COX-2 versus purified human COX-2 revealed that 5 indomethacin displayed greater inhibition of the mouse enzyme than of the human isoform (IC_{50} (mouse COX-2) ~ 350 nM; IC_{50} (human COX-2) ~ 1 μ M). On the other hand, an indomethacin-amide derivative (compound 11) was a better inhibitor of human COX-2 than of murine COX-2 (compound 11: IC_{50} (mouse COX-2) ~ 120 nM; IC_{50} (human COX-2) ~ 75 nM).

10 These results also strengthen another researcher's previous observations which suggest that COX enzymes from the rat are pharmacologically different from those from humans, as reported in Ramesha, "Human and Rat Cyclooxygenases are Pharmacologically Distinct", *Adv. Exp. Med. Biol.* (1997) Vol. 407, pp. 67-71.

15 Example IV

Testing for reduction in inflammation.

Compound 14 was tested in a standard *in vivo* assay of inflammation - the rat foot pad edema model. This assay is widely used in the pharmaceutical industry to evaluate antiinflammatory compounds. Rats were injected with 20 carrageenan, which triggers a rapid edema (swelling) within 3 hours that can be quantitatively measured by volume displacement. A single dose of

-49-

compound 14 (2 mg/kg) given orally 1 hour after carrageenan injection caused a dramatic decrease in swelling.

In these experiments, the carrageenan that was injected was in 0.1 mL of aqueous saline so that 0.1 mL volume increase was due to the injection alone. Taking this into consideration, approximately an 80-85% reduction in inflammation following treatment with compound 14 was found. For comparison, indomethacin was also tested in this assay at a dose of 2 mg/kg orally, and comparable reduction in inflammation was found.

More specifically, male Sprague-Dawley rats (150 g) received a subplantar injection of carrageenan (0.1 mL of a 1% suspension of carrageenan in sterile aqueous saline) into the right hind footpad while mildly anesthetized with methoxyflurane. At 1 hour post-injection, the rats were gavaged with 0.5 mL corn oil containing either 90 µL DMSO or 90 µL compound 14 for the doses specified below. The ipsilateral footpad volume (mL) was measured with a water displacement plethysmometer at time = 3 hours post-injection and compared to the time = 0 pre-injection paw volume for edema calculations.

For each dose, 6 rats were injected, and the results are summarized below.

-50-

<u>Compound 14</u>		
<u>Concentration (mg/mL)</u>	<u>3 hour edema (mL)</u>	<u>standard deviation</u>
0	0.87	0.1
0.2	0.55	0.04
0.5	0.47	0.07
1.0	0.39	0.03
2.0	0.38	0.07

Example V

Testing for tumor inhibition.

10 The ability of compound 11 to inhibit the growth of a human colon cancer cell line in nude mice (human tumor xenograft assay) was determined.

Following subcutaneous inoculation of the mice with the human tumor cells, the animals were injected intraperitoneally 3 times a week with vehicle alone or vehicle containing 5 mg/kg of compound 11.

15 More specifically, male athymic Nude-nu (nu/nu) mice (25 g, 6-8 weeks old, Harlan Sprague-Dawley) were injected intraperitoneally with 100 µL vehicle (5% ethanol, 5% Tween 80 in sterile aqueous PBS) or 100 µL compound 11 in vehicle immediately prior to subcutaneous implantation of 5X10⁶ HCA-7 (human colon adenocarcinoma, colony 29) cells in PBS into the dorsal surface.

20 The mice, 5 animals per group, received injections of vehicle or vehicle containing compound 11 (5 mg/kg) 3 times per week. Tumor volume was determined by external measurement with an electronic caliper.

From comparison of the growth of the tumors as summarized below, it can be seen that compound 11 significantly inhibited tumor growth.

-51-

5

<u>Day of Explant</u>	<u>Control tumor (vol. cm³)</u>	<u>compd. 11 (tumor vol. cm³)</u>
22	0.34	0.10
25	0.47	0.11
27	0.62	0.13
29	0.70	0.14
31	0.76	0.17

It will be understood that various details of the invention may be changed without departing from the scope of the invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the 10 purpose of limitation--the invention being defined by the claims.

CLAIMS

What is claimed is:

1. A method for cancer treatment in a warm blooded vertebrate animal, comprising administering to the animal a treatment effective amount sufficient to inhibit cancer of a carboxylic acid secondary amide derivative of a compound, wherein:
 - (1) the derivative is selective for inhibition of cyclooxygenase-2, and
 - (2) the compound (a) is a cyclooxygenase inhibitor but is absent selectivity for inhibition of cyclooxygenase-2 and (b) contains a carboxylic acid moiety or a pharmaceutically acceptable salt thereof.
2. The method of claim 1, wherein the cancer treatment is antiangiogenic treatment and the amount sufficient to inhibit cancer is sufficient to inhibit blood cells that feed cancer or blood vessel cells that feed cancer.
- 15 3. The method of claim 1, wherein the cancer treatment is antitumor treatment and the amount sufficient to inhibit cancer is sufficient to inhibit tumor growth.
4. The method of claim 3, further including the derivative possesses an analgesic, antiinflammatory, or antipyretic property as possessed by the compound and the treatment provides an analgesic, antiinflammatory, or antipyretic effect in the animal absent concomitant administration of another compound for providing such effect.

-53-

5. The method of claim 1, wherein the compound is a non-steroidal antiinflammatory drug.

6. The method of claim 5, wherein the non-steroidal antiinflammatory drug is selected from the group consisting of fenamic acids, 5 indoles, phenylalkanoic acids, phenylacetic acids, pharmaceutically acceptable salts thereof, and combinations thereof.

7. The method of claim 5, wherein the non-steroidal antiinflammatory drug is selected from the group consisting of indomethacin, 10 6-methoxy- α -methyl-2-naphthylacetic acid, meclofenamic acid, diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolac acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, 15 orpanoxin, pirprofen, pranoprofen, tolafenamic acid, zaltoprofen, zomopirac, pharmaceutically acceptable salts thereof, and combinations thereof.

8. The method of claim 1, wherein the secondary amide derivative is a secondary amide derivative of a non-steroidal antiinflammatory drug.

9. The method of claim 1, wherein the secondary amide derivative 20 is selected from the group consisting of secondary amide derivatives of fenamic acids, indoles, phenylalkanoic acids, phenylacetic acids, and combinations thereof.

10. The method of claim 1, wherein the secondary amide derivative is selected from the group consisting of secondary amide derivatives of indomethacin, 6-methoxy- α -methyl-2-naphthylacetic acid, meclofenamic acid, diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, 5 suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolac acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, orpanoxin, pirprofen, pranoprofen, tolfenamic acid, zaltoprofen, zompirac, and 10 combinations thereof.

11. The method of claim 1, wherein the secondary amide derivative is selected from the group consisting of indomethacin-*N*-methyl amide, indomethacin-*N*-ethan-2-ol amide, indomethacin-*N*-octyl amide, indomethacin-*N*-nonyl amide, indomethacin-*N*-(2-methylbenzyl) amide, indomethacin-*N*-(4-15 methylbenzyl) amide, indomethacin-*N*-(*(R)*-4-dimethylbenzyl) amide, indomethacin-*N*-(*(S)*-4-dimethylbenzyl) amide, indomethacin-*N*-(2-phenethyl) amide, indomethacin-*N*-(4-fluorophenyl) amide, indomethacin-*N*-(4-chlorophenyl) amide, indomethacin-*N*-(4-acetamidophenyl) amide, indomethacin-*N*-(4-methylmercapto)phenyl amide, indomethacin-*N*-(3-20 methylmercaptophenyl) amide, indomethacin-*N*-(4-methoxyphenyl) amide, indomethacin-*N*-(3-ethoxyphenyl) amide, indomethacin-*N*-(3,4,5-trimethoxyphenyl) amide, indomethacin-*N*-(3-pyridyl) amide, indomethacin-*N*-(2-chloro)pyridyl) amide, indomethacin-*N*-5-((1-ethyl)pyrazolo) amide,

-55-

indomethacin-N-(3-chloropropyl) amide, indomethacin-N-methoxycarbonylmethyl amide, indomethacin-N-2-(2-L-methoxycarbonylethyl) amide, indomethacin-N-2-(2-D-methoxycarbonylethyl) amide, indomethacin-N-(4-methoxycarbonylbenzyl) amide, indomethacin-N-(4-methoxycarbonylmethylphenyl) amide, indomethacin-N-(2-pyrazinyl) amide, indomethacin-N-2-(4-methylthiazolyl) amide, indomethacin-N-(4-biphenyl) amide, and combinations thereof.

12. The method of claim 1, wherein the treatment effective amount sufficient to inhibit cancer ranges from about 0.5 milligram to about 7.0 milligrams per kilogram of body weight of the animal per day.

13. The method of claim 1, wherein the treatment effective amount sufficient to inhibit cancer ranges from about 1.5 milligrams to about 6.0 milligrams per kilogram of body weight of the animal per day.

14. The method of claim 1, wherein the treatment effective amount sufficient to inhibit cancer ranges from about 2.0 milligrams to about 5.0 milligrams per kilogram of body weight of the animal per day.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/30220

A. CLASSIFICATION OF SUBJECT MATTER		
IPC(7) : A01N 43/88; A61K 31/40 US CL : 514/420, 419 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/420, 419		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched NONE		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) NONE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,510,368 A (LAU et al.) 23 April 1996, see abstract and columns 1-3.	1-14
Y	MCMURRY, J. Organic Chemistry, Second Edition. Pacific Grove, CA: Brooks/Cole Publishing Company, 1988, pages 742-745, especially page 744.	1-14
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "E" earlier document published on or after the international filing date "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family		
Date of the actual completion of the international search 27 MARCH 2000	Date of mailing of the international search report 04 APR 2000	
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DWAYNE C. JONES  Telephone No. (703) 308-1235	

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.